



Review Article

Circadian rhythms and inflammatory diseases of the liver and gut[☆]

Jessica M. Ferrell

Department of Integrative Medical Sciences, Northeast Ohio Medical University, Rootstown, OH, USA

ARTICLE INFO

Article history:

Received 17 May 2023

Received in revised form

24 July 2023

Accepted 14 August 2023

Keywords:

Circadian rhythm

Liver

Gut

Inflammation

Non-alcoholic fatty liver disease (NAFLD)

Alcohol-associated liver disease (AALD)

ABSTRACT

Circadian rhythms play a central role in maintaining metabolic homeostasis and orchestrating inter-organ crosstalk. Research evidence indicates that disruption to rhythms, which occurs through shift work, chronic sleep disruption, molecular clock polymorphisms, or the consumption of alcohol or high-fat diets, can influence inflammatory status and disrupt timing between the brain and periphery or between the body and the external environment. Within the liver and gut, circadian rhythms direct the timing of glucose and lipid homeostasis, bile acid and xenobiotic metabolism, and nutrient absorption, making these systems particularly susceptible to the effects of disrupted rhythms. In this review, the impacts of circadian disruption will be discussed with emphasis on inflammatory conditions affecting the liver and gut, and the potential for chronotherapy for these conditions will be explored.

© 2023 The Third Affiliated Hospital of Sun Yat-sen University. Publishing services by Elsevier B. V. on behalf of KeAi Communications Co., Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction to circadian rhythms

Circadian rhythms refer to physiological and biological processes that occur with a repeating period of approximately 24 h. These endogenous rhythms are generated and maintained by the suprachiasmatic nucleus (SCN), a cluster of approximately 20,000 neurons within the mammalian hypothalamus. Circadian rhythms serve to temporally organize cellular function and to allow for timely adaptation to changes in the external environment. Circadian rhythms are characterized by three fundamental properties: (i) they free-run in the absence of light; (ii) they are temperature-compensated, so that the rhythm remains stable over a range of physiological temperatures; and (iii) they are entrained by external stimuli. The most powerful entraining agent is environmental light.

1.1. Mammalian circadian rhythms

In humans, the endogenous circadian period is approximately 24 h 11 min, slightly longer than the solar day. Environmental light cues received by the SCN entrain the clock to external time, thus preventing daily drift and misalignment. Intrinsically photosensitive retinal ganglion cells are specialized non-image-forming cells that transmit environmental photic information to the SCN via the retinohypothalamic tract. This photic input influences the firing rate of SCN neurons, which endogenously

generate the transcriptional-translational feedback loop (TTFL) that ultimately determines molecular and cellular rhythms in the brain and periphery. The timing of photic input also influences SCN function and rhythmic behavior: light perceived early in the dark period is registered as an extension of daytime. This shifts activity of the SCN and causes downstream rhythms to occur later than expected, termed a phase delay. Conversely, light perceived later in the dark period is registered as an artificially early start of daytime, and results in a phase advance. Light perceived during the subjective day has little effect on the timing of the circadian clock in mammals, termed the “dead zone” (Fig. 1A).

The self-sustained TTFL exists in nearly all cell types and consists of a series of clock genes who products create feedback loops that regulate their own transcription (Fig. 1B). The transcription factors circadian locomotor output cycles kaput (Clock) and brain and muscle ARNT-like protein 1 (Bmal1) heterodimerize and bind to E-box elements in the Period (Per1/2/3) and Cryptochrome (Cry1/2/3) promoter regions, thus driving their transcription. Per and Cry proteins then dimerize to interfere with Clock/Bmal1 activity, which ultimately suppresses Per and Cry transcription. Per and Cry proteins are also phosphorylated by casein kinase 1, which acts as a tag for ubiquitination and degradation, thus completing a 24 h cycle. The TTFL is supplemented with accessory feedback loops that serve to modulate expression of the core clock genes. The nuclear receptors Rev-erb alpha and retinoid-related orphan receptor alpha (RORα) competitively bind the ROR-response element (RORE) in the Bmal1 promoter to suppress or induce gene transcription, respectively. This robust TTFL originates in the SCN and is propagated through neural and hormonal signals, further targeting thousands

[☆] Edited by Peiling Zhu.

E-mail address: jfranc@neomed.edu.

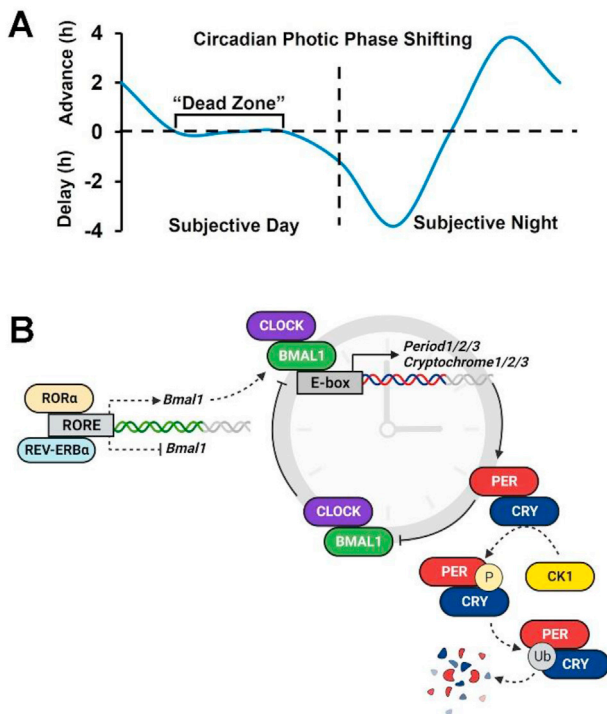


Fig. 1. The mammalian circadian clock. (A) Circadian photic phase shifting. Light perceived by the circadian clock differentially affects behavior; light perceived in the early subjective night results in a phase delay of downstream activities, while light perceived late at night results in a phase advance. Light perceived during the subjective day (termed the “dead zone”) has little effect on the circadian clock or behavior. (B) The circadian transcriptional-translational feedback loop (TTFL). The core clock mechanism is pictured in solid lines and accessory regulation is pictured in dotted lines. The core clock proteins CLOCK and BMAL1 heterodimerize at E-box regions of the Period (*PER*) and Cryptochrome (*CRY*) gene promoters, driving their transcription. *PER* and *CRY* proteins inhibit the actions of the CLOCK-BMAL1 heterodimer, ultimately reducing their own transcription. *PER* and *CRY* are phosphorylated by casein kinase 1 (CK1), which further tags these proteins for ubiquitin-mediated degradation. The accessory proteins ROR α and REV-ERB α regulate *Bmal1* transcription by competitively binding at the ROR-response element (RORE) in the *Bmal1* promoter to enhance or inhibit *Bmal1* transcription, respectively. This molecular feedback loop lasts approximately 24 h, thus completing one circadian cycle. This figure was created with BioRender.com. Abbreviations: BMAL1, brain and muscle ARNT-like protein 1; CLOCK, circadian locomotor output cycles kaput; ROR α , retinoid-related orphan receptor alpha.

of clock-controlled genes to ensure synchronization of peripheral tissues with the central clock. This TTFL also exists in nearly all mammalian tissues, with conserved homologous regulation identified in bacteria and fungi, insects, and plants.¹ Overall, this synchronization ensures organisms can adapt to dynamic cellular and physiological conditions with appropriately timed responses.

1.2. Circadian disruption in the liver and gut

Energy needs oscillate over the 24 h day, and stable circadian rhythms are required to control the timing of nearly all metabolic functions, including sleep-wake cycles, body temperature and hormone fluctuations, glucose metabolism, and inflammatory responses. In the mammalian liver, the transcriptome and proteome are regulated in a rhythmic fashion due to direct circadian regulation as well as rhythmic modulation of nuclear transport and post-translational modifications.^{2,3} This ensures, on a molecular and cellular level, that rhythmic influence over glucose and lipid metabolism, bile acid homeostasis, and xenobiotic processing results in favorably timed metabolic responses to nutrient availability. Disruption to circadian rhythms due to diet or alcohol, shift

work, or sleep loss can result in misalignment of the internal clock with the external environment, or a loss of central-to-peripheral temporal organization within an organism. This desynchronization contributes to the pathogenesis of cardiovascular and neurodegenerative diseases,^{4,5} metabolic syndrome,⁶ and many types of cancer,⁷ and underlying deregulation of immune homeostasis may also be a significant contributor.⁸ Cells of both the innate and adaptive immune systems exhibit rhythms in gene expression and function. Leukocyte circulation and infiltration oscillate in a circadian fashion, with the exact timing dependent on cell and tissue type.⁹ Circadian mediation of the immune response may be partly mediated through clock-controlled glucocorticoid release.¹⁰ Meanwhile, pro-inflammatory cytokines can disrupt timing of the molecular clock, and clock disruption can increase inflammation. The pro-inflammatory nuclear factor-kappa B (NF- κ B) was shown to bind to the E-box region of the *Bmal1* promoter, repressing its transcription and altering period and amplitude of clock gene rhythms.¹¹ Conversely, deletion of *Bmal1* in macrophages increased their motility and resulted in a “preactivation” phenotype.¹² Circadian systems, sleep duration and timing, and chronotype (individual phase preference for morning vs. evening, i.e., morning lark vs. night owl) all play roles in the maintenance of homeostasis, as well as disease pathology when these systems become dysregulated (Fig. 2). Reciprocal signaling between clocks and the immune system is involved in the pathogenesis of obesity and metabolic syndrome, gut dysbiosis, and many other diseases.^{13–15} Here, these interactions will be examined with emphasis on inflammatory liver and bowel diseases, as well as the therapeutic potential for time-based treatment of these disorders (chronotherapy).

2. Circadian rhythms in inflammatory liver diseases

2.1. Metabolic dysfunction-associated steatosis and steatohepatitis

Metabolic dysfunction-associated steatotic liver disease (MASLD), formerly known as non-alcoholic fatty liver disease (NAFLD),

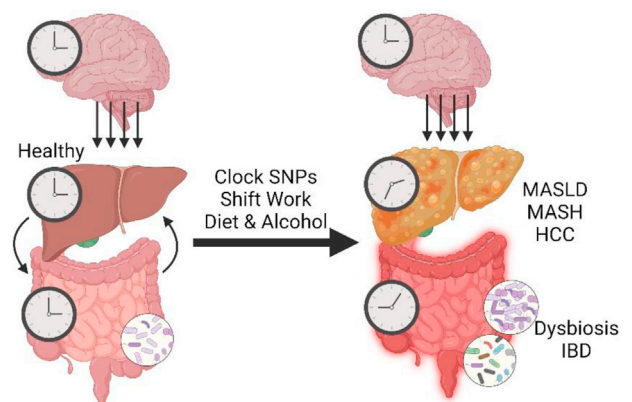


Fig. 2. Circadian disruption and pathogenesis in the liver and gut. (Left) The biological clock of the hypothalamus produces endogenous rhythms that synchronize the timing of metabolic processes in the liver and gut to the external environment and to each other. (Right) Disruptions to circadian rhythms, which can occur as clock gene single nucleotide polymorphisms (SNPs) or other genetic mutations, chronic shift work, or high-calorie diets and alcohol, desynchronize metabolic timing. This desynchronization contributes to the pathogenesis of metabolic dysfunction-associated steatotic liver disease (MASLD), metabolic dysfunction-associated steatohepatitis (MASH), and hepatocellular carcinoma (HCC) in the liver. In the gut, desynchronization alters microbe populations and can lead to dysbiosis in the form of bacterial overgrowth or disrupted microbiome composition, as well as contribute to conditions like inflammatory bowel disease (IBD). This figure was created with BioRender.com.

comprises a spectrum of disorders and is an increasing public health concern. MASLD often begins with simple steatosis (NAFL), in which more than 5% of liver weight is fat. Steatosis is estimated to occur in as much as 25% of the global population, with prevalence increasing in both women and children.^{16,17} A proportion of patients with steatosis will progress to more serious forms of MASLD, including metabolic dysfunction-associated steatohepatitis (MASH), formerly known as non-alcoholic steatohepatitis (NASH), fibrosis and cirrhosis, or hepatocellular carcinoma (HCC) and end-stage liver disease,¹⁸ making MASLD one of the leading causes of chronic liver disease. MASLD is often associated with metabolic syndrome, including obesity and type 2 diabetes, though MASLD progression can occur in lean patients independent of weight.^{19,20} Additionally, MASLD is associated with distinct changes in transcription factors in the liver, including sterol regulatory element-binding protein (Srebp)-mediated lipogenesis, carbohydrate-responsive element-binding protein (Chrebp)-maintained glucose homeostasis, and Nf- κ b-linked inflammation.²¹

Circadian disruption, often occurring in the form of long-term shift work, can also lead to liver dysfunction and contribute to MASLD pathogenesis. Night shift work was associated with increased serum alanine transaminase (ALT) independent of MASLD status,²² while another study demonstrated that chronic shift workers with MASLD had a 3.7-fold increased risk for elevated ALT compared to workers with non-shift schedules or those without MASLD.²³ Likewise, serum alkaline phosphatase was increased in shift workers even after adjusting for age, body mass index, and fasting blood sugar and cholesterol.²⁴ Another small study found that patients with MASLD exhibited poorer quality and shorter sleep coupled with shifted food intake toward nighttime, implicating a role for disrupted rhythms in MASLD.²⁵ Conversely, self-identified short sleep duration (<5 h) was associated with a higher risk of developing MASLD compared to long sleep duration (>7 h) in a study of more than 12,000 Japanese subjects.²⁶ Evening chronotype (night owl) was also associated with obesity and higher risk of MASLD in human patients.^{27–29} Though can be difficult to establish a causal pathology in human patients, results from animal studies indicate that circadian desynchrony contributes to MASLD. Clock mutant mice have a significantly increased circadian period (approximately 27–28 h) and are both hyperphagic and obese.³⁰ Even on a standard diet, these mice developed steatosis with increased serum ALT by 10 months of age, which was mediated by increased hepatic lipid uptake and synthesis coupled with reduced export.³¹ Feeding these mice a high-fat diet resulted in further significant increases in body weight with elevated ALT and thio-barbituric acid-reactive substances, an indicator of lipid peroxidation. The obese phenotype of Clock mutant mice was partially rescued with Clock gene delivery, which reduced body weight and food intake, and restored energy expenditure to wild type levels.³² Mice deficient of Bmal1 and Apoe were hyperlipidemic with increased very-low-density lipoprotein production and reduced biliary cholesterol efflux,³³ while adipocyte-specific Bmal1 knockout mice were more obese than wild type controls when fed either standard or high-fat diet.³⁴ These mice also consumed more calories during the inactive period (daytime) compared to controls, despite the groups consuming the same number of calories overall. Analysis of human clock polymorphisms corroborates data in rodent studies. Humans with CLOCK gene polymorphisms are significantly more obese and are less responsive to dietary intervention compared to non-carriers.^{35,36} In addition to CLOCK, polymorphisms in BMAL1, PER2, and CRY2 are associated with obesity, hyperglycemia, liver triglyceride content, and other disorders in humans,^{37–39} though at least one recent meta-analysis examining 13 studies with more than 17,000 subjects found that only BMAL1 variants were associated with increased risk of metabolic

syndrome.⁴⁰ Still, these studies indicate that disruption of the circadian clock, either endogenously or exogenously, likely contributes to the pathogenesis of MASLD and related metabolic disorders.

In addition to genetic clock disruption, rodent models of shift work also provide insight into the role of the peripheral clock in mediating liver homeostasis. High-fat diet-fed mice exposed to a rotating light schedule, simulating shift work, weighed more and ate more food during the inactive period compared to mice on a standard fixed light schedule. Expression of pro-inflammatory macrophages (F4/80⁺ CD11b⁺ CD11c⁺ CD206[−] cells) and interleukin-1beta (Il-1 β), Il-6, and tumor necrosis factor-alpha (Tnf- α) expression in adipose tissue were also significantly induced.⁴¹ Similarly, 8 weeks of light schedule rotation in mice caused weight gain and hepatic steatosis even on a standard chow diet, which was further exacerbated with increased ALT after feeding high-fat diet.⁴² Exposure of mice to six months of rotating light schedules caused adipocyte hypertrophy and an adipose transcriptome characterized by increased inflammation, angiogenesis, and fibrosis.⁴³ Another study found that either constant light or a rotating light schedule induced serum levels of Tnf- α , Il-6, Il-1 β , as well as serum lipids, aspartate transaminase (AST) and ALT in mice.⁴⁴

Circadian disruption can also result from social jet lag or the twice-yearly clock changes observed with Daylight Saving Time (DST) in the United States. Social jet lag, a form of chronic circadian disruption determined by calculating the difference in sleep timing on work days vs. free days, may contribute to metabolic syndrome by increasing inflammatory states. One study demonstrated that social jet lag was significantly associated with metabolic obesity and increased inflammation in humans, even after accounting for sleep duration.⁴⁵ Several other studies have demonstrated similar associations between social jet lag and obesity in both adults and children,^{46–48} and these associations remain after adjustment for confounders like age, sex, and sleep quality. However, further longitudinal research is required, as chronotype alone may also be a significant contributing factor.⁴⁷

DST is an example of circadian disruption that causes acute physiological harm. In the United States, DST is a federally mandated period in which social time is advanced 1 h in March (colloquially referred to as “spring forward”). This artificial advancement of time results in 1 h of misalignment and an average of 19 min of sleep lost every night throughout the observance of DST.⁴⁹ The “spring forward” transition from natural standard time to DST is acutely harmful and is associated with increased mortality,⁵⁰ cardiovascular morbidity,^{51,52} motor vehicle accidents,⁵³ and incidence of mood disorders.⁵⁴ Notably, many of these phenomena subsequently decrease after the autumnal transition to standard time (“fall back”), indicating these risks might be alleviated with the abolishment of DST. A recent study also identified significant increases in noninfective enteritis and colitis after the spring shift into DST,⁵⁵ suggesting effects on the gut and enteric immune system are likely understudied. As mentioned, light is the most powerful entraining agent of the circadian clock. DST artificially delays sunrise and sunset by 1 h, and late sunsets are associated with obesity, type 2 diabetes, and cardiovascular disease.^{49,56} Additionally, data indicates human rhythms do not adjust to altered timing during DST, as cortisol rhythms advance by approximately 2 min rather than 60 min.⁵⁷ Together, these studies demonstrate both physiological susceptibility to insults from circadian disruption and an inability to adjust to chronic disruption; therefore, every effort should be made to mitigate the health risks associated with disruption of circadian rhythms.

The progression of MASLD to MASH was once attributed to a “two-hit hypothesis” in which steatosis represents the first

metabolic insult and increased inflammation the second. A more accurately detailed model might be described as a “multi-hit hypothesis”, in which multiple risk factors including poor diet, obesity, type 2 diabetes or insulin resistance, oxidative stress, lipotoxicity, and microbe-derived endotoxin release and subsequent permeabilization of the gut all interact to contribute to disease progression. Circadian disruption is likely an additional hit that influences both lipid metabolism and inflammatory processes in the liver. Constant light exposure, a model for circadian disruption by light pollution, worsened insulin resistance and increased inflammation induced by a high fructose diet in rats.⁵⁸ Several clock proteins have been implicated in the disease progression of MASH, while restriction of food intake, known as intermittent fasting or time-restricted eating (TRE), may provide some benefit. Mice fed a high fructose diet were obese with increased hepatic expression of *Clock*, *Bmal1*, and *Per1/2*, and restricting access to this diet only to the active phase, one form of TRE, reversed symptoms of MASH and restored clock gene expression.⁵⁹ Non-parenchymal cells isolated from hepatocyte-specific *Rev-erba/β* double knockout mice fed a high-fat diet had disrupted rhythms in gene expression that were rescued by the deletion of *Srebp* signaling, as were rhythms in lipid metabolites, which may be important for the progression of MASH.⁶⁰ Conversely, treatment with the *Rev-erba/β* agonist SR-9009 reduced diet-induced steatosis, fibrosis, and inflammatory activity in mice.⁶¹ Several candidate drugs in trials for the treatment of MASH target clock-controlled outputs,⁶² though it's not clear that rhythmicity or dose timing is considered in trial designs. Therefore, further emphasis should be placed on determining how manipulation of the clock affects inflammatory disease progression in MASLD and MASH at both pre-clinical and clinical research levels.

2.2. Alcohol-associated liver disease (AALD)

Alcohol and rhythms exist in a complex bi-directional relationship, and alcohol consumption influences the timing of the peripheral molecular clock. Interestingly, acute application of ethanol to SCN *in vitro* slice preparations alters glutamate-induced phase changes in SCN neuronal firing activity, though ethanol alone does not alter the central clock phase.⁶³ Similarly, a study in mice fed an ethanol-containing diet demonstrated that ethanol-induced phase advances in clock gene expression in the liver, while gene expression in the SCN was not affected.⁶⁴ Mice fed an ethanol-containing diet for 4 weeks displayed dampened nighttime activity, suppression of rhythmic clock gene transcription in the liver, and phase-advancement of hepatic clock gene expression.⁶⁵ Another study utilizing a similar model showed that alcohol blunted rhythms in hepatic clock gene expression and this effect was worse in *Bmal1*-liver-specific knockout mice. These mice also had increased liver and plasma triglycerides and altered rhythms in the lipogenic transcription factors *Srebp1c* and *Chrebp*.⁶⁶ Likewise, a recent systematic review found that alcohol consumption altered rhythms of serum melatonin and cortisol in humans, which was dependent on acute vs. chronic alcohol use.⁶⁷

Disruption to rhythms is associated with propensity for alcohol consumption or addiction. When presented with a choice between ethanol or water, *Clock* mutant mice display a significantly increased preference for ethanol consumption,^{68,69} and when fed an ethanol diet these mice displayed distinct tissue-specific alterations in gene expression.⁷⁰ Many affected hepatic and colonic genes were clock-related or clock-controlled, with alcohol having only a minor effect on inflammatory gene expression in the liver and a larger effect in the colon.⁷⁰ Likewise, selective striatal ablation of *Bmal1* or *Per2* in mice increased preference for ethanol consumption.⁷¹ Chronotype in humans may also influence alcohol

consumption, with evening preference associated with increased alcohol consumption and alcohol use disorder (AUD),^{72–74} though at least one study refutes this association.⁷⁵ Therefore, additional study into clock disruption and chronotype on the influence of alcohol consumption and use disorder is warranted.

Like MASLD, AALD is a spectrum of conditions encompassing steatosis, hepatitis, fibrosis, and HCC that can result from the consumption of alcohol. The liver is the primary site of alcohol metabolism, in which ethanol is converted to acetaldehyde and acetate via NAD⁺-dependent pathways. Excess buildup of NADH leads to inhibition of fatty acid β -oxidation while alcohol itself increases hepatic fatty acid uptake. Alcohol also influences lipogenesis via upregulation of *Srebp1c* and *Chrebp*, ultimately resulting in steatosis.⁷⁶ Hepatic lipid metabolism is coupled with the sleep-wake cycle and oscillations in feeding and fasting, making the liver highly susceptible to alterations in metabolic homeostasis due to excess alcohol consumption. Ethanol was shown to weaken *Clock*-*Bmal1* protein interactions *in vitro*, while liver-specific deletion of *Bmal1* in mice resulted in more severe alcohol-induced liver injury, including increased lipid accumulation and injury attributed to mitochondrial damage.⁷⁷ Also in mice, chronic-plus-binge ethanol feeding phase-shifted and suppressed mRNA expression of hepatic *Clock*, *Bmal1*, and *Per2*, while administration of melatonin, a hormone secreted by the pineal gland, rescued these rhythms. Owing to its antioxidant activities, this study also demonstrated that melatonin activated anti-ferroptosis pathways via *Bmal1* in mice, thus mitigating ethanol-induced liver damage.⁷⁸ Another study using the same ethanol-feeding model confirmed ethanol suppressed *Bmal1* in mouse liver, and further discovered that liver-specific overexpression of *Bmal1* reduced steatosis and circulating triglycerides in mice. However, the effects on the peripheral clock were not addressed in this study.⁷⁹

2.3. Cholestatic liver diseases

Cholestatic liver disease describes a range of conditions resulting from damage to bile ducts and/or impaired bile flow into the biliary system. These disorders are typically defined as intrahepatic (involving hepatocytes or intrahepatic bile ducts) or extrahepatic (involving extrahepatic bile ducts or other blockages outside the liver). Two of the most commonly diagnosed conditions that cause cholestasis, primary biliary cirrhosis (PBC), and primary sclerosing cirrhosis (PSC), are progressive chronic disorders that can ultimately lead to serious liver disease including cirrhosis, and they may involve an autoimmune pathogenesis. Many patients with PSC may have a distinct form of inflammatory bowel disease (IBD) termed PSC-IBD, and may be at higher risk for developing colorectal cancer and death.⁸⁰ This condition is characterized by a clinical profile distinct from other forms of IBD like ulcerative colitis (UC) or Crohn's disease (CD), discussed below, with a more mild disease course and higher rates of pancolitis.⁸¹

PBC and PSC may also involve a circadian component, as mice lacking *Per2* had significant induction of inflammatory and fibrotic gene expression after bile duct ligation (BDL, a model of cholestasis) along with increased hepatic stellate cell activation which is indicative of a fibrotic state.⁸² Many patients have fatigue or sleep disorders, including delayed sleep phase cycles. A small study of patients with PBC underwent morning bright-light therapy for 2 weeks, which improved sleep quality and timing as well as robustness of melatonin rhythms.⁸³ Research in animal models indicates melatonin itself may have direct role in pathogenesis of cholestasis. Knockout of the melatonin 1A receptor in mice with BDL resulted in reduction of liver fibrosis and inflammation, while knockout of the melatonin 1B receptor worsened the BDL phenotype. Additionally, the beneficial effects of melatonin administration

were limited to the 1B knockout mice, indicating that melatonin affects cholestatic responses via the 1A receptor.⁸⁴ Another study demonstrated a worsened BDL phenotype after pinealectomy in rats, which was recapitulated in human hepatic stellate cells exposed to cholangiocytes from those rats.⁸⁵ Finally, a recent clinical trial examined the efficacy of melatonin in relieving cholestatic pruritus and found melatonin improved both pruritus and sleep disturbances in patients with chronic liver disease.⁸⁶ Melatonin may represent a potential therapy for cholestatic liver diseases, though further research is required to determine the effects of long-term melatonin administration on the circadian system in the brain, liver, and elsewhere.

2.4. HCC

The global landscape of primary liver cancer is changing with the advent of successful anti-viral treatments for hepatitis B and C, coupled with the rise in alcohol- and MASH-related causes. Alcohol was estimated to contribute to approximately 20% of liver cancer-related deaths in 2019,⁸⁷ with MASH and alcohol having the highest age-standardized death rates.⁸⁸ HCC accounts for approximately 75% of primary liver cancers and is often co-morbid with other liver diseases.⁸⁹

Circadian disruption is linked to increased risk of cancer via mechanisms involving disruption of the circadian machinery, as well as induction of oncogenes and inhibition of tumor suppressor genes. In 2007, the International Agency for Research on Cancer designated shift work involving circadian disruption as a Class 2A (probable) human carcinogen, and that designation was maintained at a review conducted in 2019.⁹⁰ Position-in-time-zone studies provide an interesting perspective on the cancer-causing effects of permanent circadian disruption. Time zones are meant to be centered on longitudinal meridians, with the sun overhead at noon and the eastern and western edges of time zones no more than 30 min misaligned from environmental time. However, manipulation of time zone boundaries in the United States resulted in western edges of time zones that are misaligned by 60 min or more. This leads to misalignment of social vs. environmental time and is another form of chronic circadian disruption. Living at the western edge of a time zone in the United States significantly increases risk (rate ratio ~1.1 per 5° longitude difference) for liver cancer/HCC even after adjustment for obesity and lifestyle factors,^{91,92} demonstrating a need for systemic monitoring of circadian health at the public health level. In fact, people living at the western edges of time zones in the United States may serve as analogues to study the detrimental effects of DST.

Patients with HCC may have disruptions in genes involved in circadian homeostasis. The clock genes *PER1* and *RORα* were differentially expressed in HCC tumor samples compared to healthy samples, and reduced expression of these genes was associated with poorer survival. Meanwhile, methylation levels of *CRY2* and *RORα* were significantly higher in HCC samples than in healthy samples.⁹³ Another study associated increased expression of casein kinase delta, an accessory regulator of the molecular clock, with reduced overall survival in patients with HCC.⁹⁴ At a mechanistic level, chronic circadian disruption affects glucose, lipid, and bile acid homeostasis, xenobiotic metabolism, and urea disposal. Dysregulation of these processes likely contributes to the progression of MASLD/MASH to HCC. Chronic circadian shifting that simulated jet lag for 90 weeks induced MASLD in nearly 100% of wild type mice, and HCC in nearly 9% of mice. This was attributed to bile acid-mediated activation of the nuclear receptor constitutive androstane receptor, as chronic circadian disruption in *Car*^{-/-} mice induced liver clock disruption but failed to initiate inflammatory and oncogenic pathways.⁹⁵ Knockdown of either *Clock* or *Bmal1*

inhibited tumor growth *in vitro*, and HCC tumor growth was inhibited in mice transplanted with Hep3B cell xenografts lacking *Clock* or *Bmal1*.⁹⁶ *Per2* mutant mice with diethylnitrosamine (DEN)-induced HCC had nearly four times the number of tumors compared to wild type controls, with increased inflammation and apoptosis that occurred prior to the onset of carcinogenesis.⁹⁷ Melatonin is secreted in response to SCN neuronal regulation, which then feeds back to the SCN and other brain regions to synchronize rhythms and promote sleep. It's also a powerful antioxidant that has wide-ranging benefits against cellular damage from ischemia, heavy metals, and cancer.⁹⁸ Melatonin treatment reduced hepatocyte proliferation and oxidative stress while inducing apoptosis in DEN-treated rats.⁹⁹ Another study demonstrated that melatonin treatment in mice ameliorated DEN-induced changes in hepatic clock genes, including restoration of *Per* and *Cry* expression that were previously associated with poor outcome. This study also showed that melatonin alone, or in conjunction with the Rev-erbα agonist SR-9009, reduced hepatic cell proliferation *in vitro* and reduced expression of cyclin D1 and c-Myc.¹⁰⁰ Related, single nucleotide polymorphisms (SNPs) of the human melatonin receptor 1A subtype were associated with increased risk for HCC and increased metastasis in patients with HCC.¹⁰¹ Altogether, these studies provide a basis for further investigation into how disrupted rhythms contribute to hepatic disease and demonstrate how targeting rhythmic processes represents a critically under-explored aspect to treatment of disease (discussed below in chronotherapy).

3. Circadian rhythms in IBD

Like MASLD/MASH, IBD comprises a spectrum of diseases with the two most common forms being CD and UC. Studies point to increasing prevalence of IBD in both industrialized and developing nations, with an estimated 6.8 million people globally already living with some form of IBD.¹⁰² The precise cause(s) of IBD/CD/UC are mostly unknown, though genetic predisposition and innate immune problems are thought to be main contributing factors with diet, medications, infections, and other environmental factors serving as additional non-causal triggers. Both CD and UC are characterized by chronic inflammation of the gastrointestinal (GI) tract: in CD, inflammation can occur at any point in the GI tract, though it tends to be common in the distal small intestine and proximal colon, while in UC it is most often localized to the large intestine. Both conditions involve complex intestinal barrier dysfunction resulting in bacterial infiltration and dysbiosis, or disruption to the gut microbiome that can involve changes in composition or metabolic activities of the resident bacteria. This results in clinical presentations of diarrhea, weight loss, abdominal pain, and increased circulating pro-inflammatory cytokines including TNF-α.¹⁰³

3.1. CD and UC

The intestinal barrier is a critical component to maintaining gut homeostasis, and permeability of this barrier occurring from altered tight junctions or reduced mucin protection is associated with disease states. Circadian rhythms may play a role in the regulation of permeability, as the Rev-erbα agonist SR-9009 improved lipopolysaccharide (LPS)-induced permeability through the induction of tight junction proteins, including occluding and claudin 1.¹⁰⁴ Mucin production and degradation also varies rhythmically in mice, with bacterial proximity to the mucosal surface increased during the active (dark) phase, thus regulating the depth of bacterial invasion in a time-dependent manner.¹⁰⁵ This study also elegantly demonstrated that rhythmic bacterial adherence, rather than colony numbers alone, contributed to rhythmic gene

expression in the colon. However, total fecal bacterial load was also shown to oscillate rhythmically in C57BL/6J mice, and this rhythmicity was lost in *Bmal1* knockout mice.¹⁰⁶ A high-fat diet fed to mice resulted in dampened rhythmic bacterial content at the phyla level, which was partially restored by TRE,¹⁰⁷ indicating a complex relationship between direct and indirect circadian control of the gut microbiome that deserves further study.

Circadian rhythm disruption, including sleep loss and shift work,^{108,109} are linked to IBD. One prospective cohort study linked short sleep duration (self-reported <5 h/night) and daytime napping with increased risk for CD or UC after adjustment for age, sex, alcohol consumption, physical activity, and other factors.¹¹⁰ Another small study demonstrated that patients with IBD had more sleep debt and social jet lag.¹¹¹ One small study conducted in 2015 concluded that patients with CD had a similar sleep duration to healthy controls but more post-onset awakenings and worse sleep quality as measured by wrist actigraphy.¹¹² Limitations of many of these studies are the self-reported nature of sleep timing and quality in patients with IBD and the correlative nature of the associations (i.e., do sleep disturbances worsen IBD, do IBD symptoms worsen sleep, or is there a feed-forward cycle in which both occur?), highlighting a major need for more objective quantification of the associations between disrupted rhythms, sleep, and IBD.

3.2. Alcohol-associated dysbiosis

Alcohol consumption alters the microbiome in both rodents and humans, and alcohol-associated dysbiosis is associated with altered immunity, liver disease, and neurological disorders.^{113,114} Alcohol-induced dysbiosis in mice was found due to TNF- α -dependent dysfunction of the intestinal barrier,¹¹⁵ though it is likely that many other metabolic factors also contribute. *Clock*-mutant mice had lower microbial diversity compared to wild type controls, and when fed an alcohol diet these mice had further alterations in community structure and dominance by *Clostridium* and *Allobaculum*.¹¹⁶ In humans, plasma short chain fatty acids, important for intestinal barrier function, displayed a diurnal rhythm which was lost with either alcohol consumption or night-shift work.¹¹⁷ Night shift workers who drank alcohol consecutively for 7 days also had increased colonic permeability which was not recapitulated in day workers who drank alcohol.¹¹⁸ Melatonin may also play a role, as one small study demonstrated circulating melatonin was reduced in patients with AUD which was inversely correlated with severity of intestinal permeability.¹¹⁹ A recent study examined rhythmicity of the gut microbiome in patients with AUD. Overall, alcohol use reduced the relative abundance of 15 genera and 16 species, and when sampled over 24 h it was found that the microbiome of patients with AUD had more pathogenic strains with daily oscillatory patterns (including *Cyanobacteria* and *Pseudomonadaceae*) and fewer beneficial strains that oscillated (including *Prevotella*).¹²⁰ These changes in rhythmicity were also dependent on amount of alcohol consumed and duration of AUD, which could further affect stability of microbiome populations or favor growth of bacteria capable of metabolizing ethanol.¹²¹

4. Chronotherapy for the liver and gut

Chronotherapy refers to exploiting endogenous rhythms to enhance disease treatment. This can occur through direct targeting of the circadian machinery or via indirect manipulation of the rhythms that control the absorption, distribution, metabolism, or excretion of existing drugs. Chronotherapy is already utilized for the treatment of hypertension,¹²² cancer,¹²³ and depression,¹²⁴ and research suggests chronotherapy could be useful as adjunct treatments for MASLD, MASH, and HCC. One simple example is the

use of simvastatin, a cholesterol-reducing medication that inhibits hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme that synthesizes cholesterol. HMG-CoA reductase exhibits a diurnal rhythm in transcription and activity in the liver, with cholesterol synthesis typically peaking in the middle of the night. Simvastatin, with a half-life of approximately 2–3 h, is routinely prescribed with a recommendation to be taken in evening, maximizing its effects when HMG-CoA reductase is highly active. Indeed, studies examining the effectiveness of simvastatin time-of-administration determined greater reductions in low-density lipoprotein cholesterol with night-administrated simvastatin compared to daytime administration.¹²⁵ Other statins with longer half-lives may be taken at any time, while controlled-release simvastatin was shown to be effective both in the morning and evening.¹²⁵

TRE is growing in popularity as a mechanism for weight loss, as it focuses on meal timing rather than caloric intake. Animal models provide strong evidence of the benefits of TRE. Mice fed a high-fat diet *ad libitum* gained more weight than mice eating an isocaloric diet restricted only to the active period. These TRE mice also had reduced hepatic triglycerides and steatosis score, and circadian expression of sirtuin 1, a major metabolic regulator, was restored to that of chow-fed mice.¹²⁶ Another study showed that after 26 weeks of *ad libitum* high-fat diet-feeding, just two weeks of isocaloric TRE decreased hepatic inflammation prior to any changes in body weight.¹²⁷ The beneficial effects of TRE may require fibroblast growth factor 21 (Fgf21), a liver-produced hormone implicated in the improvement of metabolic syndrome. TRE increased serum Fgf21 in high-fat diet-fed mice, and liver-specific *Fgf21* knockout mice were resistant to the beneficial effects of TRE.¹²⁸

Evidence in humans is supportive of a role for TRE in improving metabolic outcomes, though most studies are lacking in sample size and generalizability. A randomized control trial demonstrated that either a 4- or 6-h daytime “eating window”, in which patients could eat *ad libitum*, produced equally significant weight loss compared to control participants who ate *ad libitum* over 24 h. This was accompanied by reductions in plasma markers of oxidative stress, likely related to improved insulin resistance, while plasma markers of inflammation were unchanged.¹²⁹ Another small study demonstrated in healthy non-obese individuals that 8 h TRE increased high-density lipoprotein cholesterol and reduced C-reactive protein and total nitrate/nitrite compared to individuals eating *ad libitum* despite equal caloric intake.¹³⁰ When combined with a low-sugar diet, 8 h TRE reduced body weight, body fat, serum ALT, AST and gamma glutamyl transferase, as well as fibrosis score in patients with MASLD.¹³¹

The efficacy and toxicity of drugs can vary over the 24 h day, as can their metabolic actions. The metabolic functions of hepatic detoxification enzymes and transporters that process these drugs are also regulated, in part, by circadian rhythms. This presents an opportunity to pharmaceutically target drug metabolism to (i) maximize efficacy of drugs that are administered, which can lead to (ii) minimizing off-target side effects by providing the lowest effective dose at a maximally effective time (Fig. 3). This requires examination of the molecular, cellular, and tissue-level processes that contribute to xenobiotic and metabolic processing under a circadian lens.

Cell cycle proliferation and DNA repair are influenced by molecular rhythms, and time-of-treatment represents a novel approach to cancer drug development.¹³² Chronomodulated administration of a combination of oxaliplatin, 5-fluorouracil, and folinic acid for colorectal cancer increased response rate by 76% and reduced toxic events compared to constant-rate infusion of the same drugs.¹³³ Limited chronotherapy studies have been conducted for the treatment of lung, head and neck cancers, glioma, and

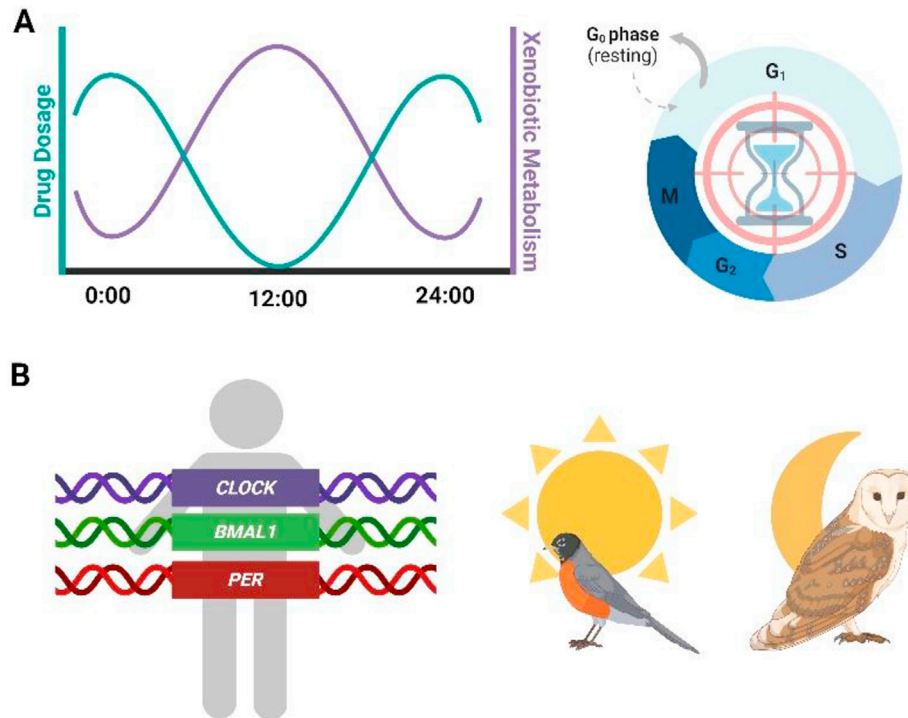


Fig. 3. Chronotherapy for drug development and disease treatment. Chronotherapy can take place in the form of generalized targeting or personalized medicine. (A) Administration of drugs at opportune times takes advantage of maximal efficiency of the xenobiotic enzymes that process those drugs, potentially resulting in lower effective doses required and reduction of side effects. Pre-existing drugs can be time-tested and optimized for better therapeutic results. Meanwhile, time-targeting of the cell cycle or circadian clock in HCC and other cancers can maximize cytotoxic effects of chemotherapeutic agents while minimizing damage to healthy tissue. (B) Progress in monitoring and understanding of patient genotype and detection of clock polymorphisms, as well as assessment of individual chronotype, will improve disease diagnosis and provide novel therapeutic targets. This figure was created with [BioRender.com](https://www.biorender.com). Abbreviations: BMAL1, brain and muscle ARNT-like protein 1; CLOCK, circadian locomotor output cycles kaput; HCC, hepatocellular carcinoma; PER, Period.

others, though overall the concept remains poorly understudied.¹³⁴ The most common curative pathway for HCC is surgical resection followed by chemotherapy and/or radiation. Recurrence rates are high and often confounded with additional underlying conditions including alcohol consumption, viral infection or cirrhosis, coupled with the problem of chemotherapeutic agents demonstrating lower efficacy with more drug resistance in HCC than in other cancers.¹³⁵ In a c-Myc mouse model of HCC, Ki67-positive cells, a marker of hepatocyte proliferation, were highest in mid-day and mid-night.¹³⁶ Likewise, mice with DEN-induced HCC had increased cell proliferation and DNA breaks at the end of the active period, and radiation treatment applied at this time minimally affected leukocyte counts. Importantly, this timing of treatment also significantly reduced the incidence of double-strand DNA breaks in non-tumor control mice while simultaneously exerting the anticipated cellular damage in HCC mice.¹³⁷ These data provide support for further studies investigating the time-dependent efficacy of anti-mitotic cancer drugs, which could minimize damage to healthy tissues while still providing effective chemotherapy.

Melatonin has been shown to be beneficial in the treatment of HCC, though whether these effects are mediated more through the modulation of circadian rhythms or the function of melatonin as an antioxidant is not entirely clear. A recent systematic review identified studies in which melatonin, by itself or in combination with additional chemotherapeutic agents for liver cancers, inhibited proliferation and tumor growth, induced apoptosis and anti-oxidant activities, and affected the immune and circadian systems, ultimately concluding that melatonin may be beneficial for the prevention and treatment of HCC and cholangiocarcinoma.¹³⁸ In mice with DEN-induced HCC, long-term

melatonin treatment prevented HCC-induced changes in hepatic clock gene expression and induced expression of the tumor suppressor proteins p21 and p53.¹⁰⁰ Melatonin was also shown to enhance the cytotoxic effects of sorafenib, a first-line chemotherapeutic for HCC, by increasing apoptosis *in vitro*,^{139,140} which was recapitulated in rats with DEN-induced HCC *in vivo*.¹⁴¹ As mentioned, melatonin administration restored clock gene expression that was altered in HCC,¹⁰⁰ while several small studies in patients with MASLD demonstrated that melatonin improved liver function and steatosis scores, reduced serum AST and ALT, and reduced circulating pro-inflammatory cytokines.^{142–144} Therefore, further consideration should be given to both direct and indirect modulation of the circadian clock for the treatment of chronic liver diseases.

Chronotherapy can also apply to chronic gut disorders. First line treatments for CD are typically anti-inflammatory agents, including glucocorticoids, azathioprine, and methotrexate. Time-dependent use of these agents has been demonstrated for the treatment of rheumatoid arthritis,^{145,146} but evidence for the chronotherapy of IBD is lacking. A small crossover trial performed in patients with inactive IBD showed that morning administration of 6-mercaptopurine and its prodrug azathioprine resulted in significantly increased levels of therapeutic metabolites and reduced levels of toxic metabolites associated with side effects compared to administration of the same drugs and doses at other times.¹⁴⁷ In a mouse model of colitis, berberine was shown to inhibit *Bmal1* promoter activity through the activation of Rev-erb α . This study also demonstrated that administration of berberine near the end of the active phase of mice reduced inflammatory markers of disease compared to administration early in the active phase.¹⁴⁸ Animal

studies have shown beneficial effects of melatonin for the treatment of colitis in rodents, though it appears time-dependent effects were not examined. In Toll-like receptor 4 knockout mice with colitis, daily melatonin induced the production of antimicrobial peptides in colon tissue and restored the species richness and diversity indices of the microbiome.¹⁴⁹ Another study investigated changes in the microbiota following melatonin treatment in colitis mice and found increased abundance of the probiotic *Bifidobacterium*, while fecal microbiota transplant from melatonin-treated mice reduced Tnf- α expression and increased tight junction protein expression in the colon of colitis mice.¹⁵⁰ These preliminary studies form the basis for further investigation into the benefits of chronotherapy for IBD, given that circadian rhythms regulate gut motility, nutrient absorption, gut immunity, and the microbiota itself.¹⁵¹

5. Conclusions and future perspectives

Circadian rhythms serve to synchronize an organism to its external environment, allowing for anticipation of daily needs and adaptation to external stimuli. These rhythms are grounded in a conserved molecular clock that exists in nearly all tissues and cells, highlighting the importance of temporal organization at the cellular and organismal level. Disruptions to these rhythms are increasingly recognized in the progression and pathology of inflammation-related diseases, including MASLD/MASH, cholangitis, HCC, AALD, and IBD. As shift work, sleep loss, and late-night activities progressively infiltrate daily life, the physiological consequences of these disruptions will need to be addressed with research that takes circadian rhythms into account.

Questions remain unanswered with respect to implementing chronotherapy in clinical practice. Currently, clinical trials are underway for the chronotherapeutic treatment of UC (NCT05213234), IBD (NCT04304950), and AUD (NCT05684094), and several trials are examining chronotypes (NCT02895282, NCT04665336) and TRE (NCT05031429, NCT04618133) for obesity and weight loss. Still, these and other trials that incorporate rhythms into interventions and outcomes comprise only a small percentage of total active trials. The influence of circadian rhythms in healthy and diseased physiology is still understudied, and the opportunity for time-targeted treatment is even less recognized. A study in 2014 indicated that more than half of the best-selling drugs in the United States targeted a product of a circadian gene, and nearly half of those drugs had half-lives short enough to be amenable to modulation of the time of administration, i.e., chronotherapy.¹⁵² Can currently-approved drugs have more favorable outcomes by studying time-of-administration? What influence do circadian rhythms have in disease diagnosis (e.g., time of biopsy, rhythms of biomarkers)? Can modulation of metabolic rhythms curtail the projected increased incidence of obesity, type 2 diabetes, and MASLD/MASH around the world? More funding and pre-clinical research are needed to answer these fundamental questions and to make personalized and chronotherapeutic medicine a clinical reality.

Author's contributions

Jessica M. Ferrell developed the concept and wrote the manuscript. The author read and approved the final version of this manuscript.

Declaration of competing interest

The author declares that there is no conflicts of interest.

References

- Li W, Wang Z, Cao J, Dong Y, Chen Y. Perfecting the life clock: the journey from PTO to TTFL. *Int J Mol Sci*. 2023;24:2402. <https://doi.org/10.3390/ijms24032402>.
- Hurni C, Weger BD, Gobet C, Naef F. Comprehensive analysis of the circadian nuclear and cytoplasmic transcriptome in mouse liver. *PLoS Genet*. 2022;18:e1009903. <https://doi.org/10.1371/journal.pgen.1009903>.
- Green CB. Circadian posttranscriptional regulatory mechanisms in mammals. *Cold Spring Harb Perspect Biol*. 2018;10:a030692. <https://doi.org/10.1101/cshperspect.a030692>.
- Rabinovich-Nikitin I, Lieberman B, Martino TA, Kirshenbaum LA. Circadian-regulated cell death in cardiovascular diseases. *Circulation*. 2019;139:965–980. <https://doi.org/10.1161/CIRCULATIONAHA.118.036550>.
- Lananna BV, Musiek ES. The wrinkling of time: aging, inflammation, oxidative stress, and the circadian clock in neurodegeneration. *Neurobiol Dis*. 2020;139:104832. <https://doi.org/10.1016/j.nbd.2020.104832>.
- Chaput JP, McHill AW, Cox RC, et al. The role of insufficient sleep and circadian misalignment in obesity. *Nat Rev Endocrinol*. 2023;19:82–97. <https://doi.org/10.1038/s41574-022-00747-7>.
- Pariollaud M, Lamia KA. Cancer in the fourth dimension: what is the impact of circadian disruption? *Cancer Discov*. 2020;10:1455–1464. <https://doi.org/10.1158/2159-8290.CD-20-0413>.
- Baxter M, Ray DW. Circadian rhythms in innate immunity and stress responses. *Immunology*. 2020;161:261–267. <https://doi.org/10.1111/imm.13166>.
- Pick R, He W, Chen CS, Scheiermann C. Time-of-day-dependent trafficking and function of leukocyte subsets. *Trends Immunol*. 2019;40:524–537. <https://doi.org/10.1016/j.it.2019.03.010>.
- Shimba A, Ikuta K. Glucocorticoids regulate circadian rhythm of innate and adaptive immunity. *Front Immunol*. 2020;11:2143. <https://doi.org/10.3389/fimmu.2020.02143>.
- Shen Y, Endale M, Wang W, et al. NF- κ B modifies the mammalian circadian clock through interaction with the core clock protein BMAL1. *PLoS Genet*. 2021;17:e1009933. <https://doi.org/10.1371/journal.pgen.1009933>.
- Kitchen GB, Cunningham PS, Poolman TM, et al. The clock gene Bmal1 inhibits macrophage motility, phagocytosis, and impairs defense against pneumonia. *Proc Natl Acad Sci U S A*. 2020;117:1543–1551. <https://doi.org/10.1073/pnas.1915932117>.
- Kolbe I, Oster H. Chronodisruption, metabolic homeostasis, and the regulation of inflammation in adipose tissues. *Yale J Biol Med*. 2019;92:317–325.
- Bishehsari F, Voigt RM, Keshavarzian A. Circadian rhythms and the gut microbiota: from the metabolic syndrome to cancer. *Nat Rev Endocrinol*. 2020;16:731–739. <https://doi.org/10.1038/s41574-020-00427-4>.
- Mashaqi S, Gozal D. "Circadian misalignment and the gut microbiome. A bidirectional relationship triggering inflammation and metabolic disorders"-a literature review. *Sleep Med*. 2020;72:93–108. <https://doi.org/10.1016/j.sleep.2020.03.020>.
- Arshad T, Golabi P, Paik J, Mishra A, Younossi ZM. Prevalence of nonalcoholic fatty liver disease in the female population. *Hepatol Commun*. 2018;3:74–83. <https://doi.org/10.1002/hep4.1285>.
- Lazarus JV, Mark HE, Anstee QM, et al. Advancing the global public health agenda for NAFLD: a consensus statement. *Nat Rev Gastroenterol Hepatol*. 2022;19:60–78. <https://doi.org/10.1038/s41575-021-00523-4>.
- Kumar R, Priyadarshi RN, Anand U. Non-alcoholic fatty liver disease: growing burden, adverse outcomes and associations. *J Clin Transl Hepatol*. 2020;8:76–86. <https://doi.org/10.14218/JCTH.2019.00051>.
- Younes R, Govaere O, Petta S, et al. Caucasian lean subjects with non-alcoholic fatty liver disease share long-term prognosis of non-lean: time for reappraisal of BMI-driven approach? *Gut*. 2022;71:382–390. <https://doi.org/10.1136/gutjnl-2020-322564>.
- Zou B, Yeo YH, Nguyen VH, Cheung R, Ingelsson E, Nguyen MH. Prevalence, characteristics and mortality outcomes of obese, nonobese and lean NAFLD in the United States, 1999–2016. *J Intern Med*. 2020;288:139–151. <https://doi.org/10.1111/joim.13069>.
- Steensels S, Qiao J, Ersoy BA. Transcriptional regulation in non-alcoholic fatty liver disease. *Metabolites*. 2020;10:283. <https://doi.org/10.3390/metabo10070283>.
- Wang F, Zhang L, Wu S, et al. Night shift work and abnormal liver function: is non-alcohol fatty liver a necessary mediator? *Occup Environ Med*. 2019;76:83–89. <https://doi.org/10.1136/oemed-2018-105273>.
- Lin YC, Chen PC. Persistent rotating shift work exposure is a tough second hit contributing to abnormal liver function among on-site workers having sonographic fatty liver. *Asia Pac J Public Health*. 2015;27:NP1765–NP1774. <https://doi.org/10.1177/1010539512469248>.
- Khosravipour M, Shah Mohammadi M. The effects of exposure to night shift work on liver function: a cross-sectional study with emphasis of alkaline phosphatase enzyme. *Chronobiol Int*. 2020;37:142–145. <https://doi.org/10.1080/07420528.2019.1684936>.
- Bernsmeier C, Weisskopf DM, Pflueger MO, et al. Sleep disruption and daytime sleepiness correlating with disease severity and insulin resistance in non-alcoholic fatty liver disease: a comparison with healthy controls. *PLoS One*. 2015;10:e0143293. <https://doi.org/10.1371/journal.pone.0143293>.
- Okamura T, Hashimoto Y, Hamaguchi M, Obora A, Kojima T, Fukui M. Short sleep duration is a risk of incident nonalcoholic fatty liver disease: a

- population-based longitudinal study. *J Gastrointest Liver Dis.* 2019;28:73–81. <https://doi.org/10.15403/jgld.2014.1121.281.alc>.
27. Maukonen M, Kanerva N, Partonen T, et al. The associations between chronotype, a healthy diet and obesity. *Chronobiol Int.* 2016;33:972–981. <https://doi.org/10.1080/07420528.2016.1183022>.
 28. Fan H, Liu Z, Zhang X, et al. Investigating the association between seven sleep traits and nonalcoholic fatty liver disease: observational and Mendelian randomization study. *Front Genet.* 2022;13:792558. <https://doi.org/10.3389/fgene.2022.792558>.
 29. Vetrani C, Barrea L, Verde L, et al. Evening chronotype is associated with severe NAFLD in obesity. *Int J Obes (Lond).* 2022;46:1638–1643. <https://doi.org/10.1038/s41366-022-01159-3>.
 30. Turek FW, Joshu C, Kohsaka A, et al. Obesity and metabolic syndrome in circadian Clock mutant mice. *Science.* 2005;308:1043–1045. <https://doi.org/10.1126/science.1108750>.
 31. Pan X, Queiroz J, Hussain MM. Nonalcoholic fatty liver disease in CLOCK mutant mice. *J Clin Invest.* 2020;130:4282–4300. <https://doi.org/10.1172/JCI132765>.
 32. Meyer-Kovac J, Kolbe I, Ehrhardt L, et al. Hepatic gene therapy rescues high-fat diet responses in circadian Clock mutant mice. *Mol Metab.* 2017;6:512–523. <https://doi.org/10.1016/j.molmet.2017.03.008>.
 33. Pan X, Bradford CA, Hussain MM. Global and hepatocyte-specific ablation of Bmal1 induces hyperlipidaemia and enhances atherosclerosis. *Nat Commun.* 2016;7:13011. <https://doi.org/10.1038/ncomms13011>.
 34. Paschos GK, Ibrahim S, Song WL, et al. Obesity in mice with adipocyte-specific deletion of clock component Arntl. *Nat Med.* 2012;18:1768–1777. <https://doi.org/10.1038/nm.2979>.
 35. Garaulet M, Corbalán MD, Madrid JA, et al. CLOCK gene is implicated in weight reduction in obese patients participating in a dietary programme based on the Mediterranean diet. *Int J Obes (Lond).* 2010;34:516–523. <https://doi.org/10.1038/ijo.2009.255>.
 36. Riestra P, Gebreab SY, Xu R, et al. Circadian CLOCK gene polymorphisms in relation to sleep patterns and obesity in African Americans: findings from the Jackson heart study. *BMC Genet.* 2017;18:58. <https://doi.org/10.1186/s12863-017-0522-6>.
 37. Yang YD, Zeng Y, Li J, et al. Association of BMAL1 clock gene polymorphisms with fasting glucose in children. *Pediatr Res.* 2023;94:653–659. <https://doi.org/10.1038/s41309-023-02467-8>.
 38. Fang Z, Zhu L, Zhang T, Jin Y, Chen Y, Yao Y. Sex-specific genetic association of brain and muscle Arnt-like protein-1 (BMAL1) and obesity in Chinese youth. *Obes Res Clin Pract.* 2022;16:464–469. <https://doi.org/10.1016/j.orcp.2022.10.008>.
 39. Garaulet M, Corbalán-Tutau MD, Madrid JA, et al. PERIOD2 variants are associated with abdominal obesity, psycho-behavioral factors, and attrition in the dietary treatment of obesity. *J Am Diet Assoc.* 2010;110:917–921. <https://doi.org/10.1016/j.jada.2010.03.017>.
 40. Škrlec I, Talapko J, Džijan S, Cesar V, Lazić N, Lepeduš H. The association between circadian clock gene polymorphisms and metabolic syndrome: a systematic review and meta-analysis. *Biology (Basel).* 2021;11:20. <https://doi.org/10.3390/biology11010020>.
 41. Kim SM, Neundorff N, Alaniz RC, Sun Y, Chapkin RS, Earnest DJ. Shift work cycle-induced alterations of circadian rhythms potentiate the effects of high-fat diet on inflammation and metabolism. *FASEB J.* 2018;32:3085–3095. <https://doi.org/10.1096/fj.201700784R>.
 42. Christie S, Vincent AD, Li H, et al. A rotating light cycle promotes weight gain and hepatic lipid storage in mice. *Am J Physiol Gastrointest Liver Physiol.* 2018;315:G932–G942. <https://doi.org/10.1152/ajpgi.00020.2018>.
 43. Xiong X, Lin Y, Lee J, et al. Chronic circadian shift leads to adipose tissue inflammation and fibrosis. *Mol Cell Endocrinol.* 2021;521:111110. <https://doi.org/10.1016/j.mce.2020.111110>.
 44. Sun Z, Li L, Yan Z, et al. Circadian rhythm disorders elevate macrophages cytokines release and promote multiple tissues/organs dysfunction in mice. *Physiol Behav.* 2022;249:113772. <https://doi.org/10.1016/j.physbeh.2022.113772>.
 45. Parsons MJ, Moffitt TE, Gregory AM, et al. Social jetlag, obesity and metabolic disorder: investigation in a cohort study. *Int J Obes (Lond).* 2015;39:842–848. <https://doi.org/10.1038/ijo.2014.201>.
 46. Li TM, Pien LC, Cheng WJ. Direction and magnitude of social jetlag are associated with obesity: results from a sample of hospital employees. *Chronobiol Int.* 2022;39:1242–1248. <https://doi.org/10.1080/07420528.2022.2090953>.
 47. McMahon DM, Burch JB, Youngstedt SD, et al. Relationships between chronotype, social jetlag, sleep, obesity and blood pressure in healthy young adults. *Chronobiol Int.* 2019;36:493–509. <https://doi.org/10.1080/07420528.2018.1563094>.
 48. Higgins S, Stoner L, Black K, et al. Social jetlag is associated with obesity-related outcomes in 9–11-year-old children, independent of other sleep characteristics. *Sleep Med.* 2021;84:294–302. <https://doi.org/10.1016/j.sleep.2021.06.014>.
 49. Giuntella O, Mazzonna F. Sunset time and the economic effects of social jetlag: evidence from US time zone borders. *J Health Econ.* 2019;65:210–226. <https://doi.org/10.1016/j.jhealeco.2019.03.007>.
 50. Poteser M, Moshhammer H. Daylight Saving Time transitions: impact on total mortality. *Int J Environ Res Public Health.* 2020;17:1611. <https://doi.org/10.3390/ijerph17051611>.
 51. Chudow JJ, Dreyfus I, Zaremski L, et al. Changes in atrial fibrillation admissions following daylight saving time transitions. *Sleep Med.* 2020;69:155–158. <https://doi.org/10.1016/j.sleep.2020.01.018>.
 52. Manfredini R, Fabbian F, Cappadona R, et al. Daylight Saving Time and acute myocardial infarction: a meta-analysis. *J Clin Med.* 2019;8:404. <https://doi.org/10.3390/jcm8030404>.
 53. Fritz J, VoPham T, Wright KP Jr, Vetter C. A chronobiological evaluation of the acute effects of Daylight Saving Time on traffic accident risk. *Curr Biol.* 2020;30:729–735 (e2). <https://doi.org/10.1016/j.cub.2019.12.045>.
 54. Johnson KG, Malow BA. Daylight Saving Time: neurological and neuropsychological implications. *Curr Sleep Medicine Rep.* 2022;8:86–96. <https://doi.org/10.1007/s40675-022-00229-2>.
 55. Zhang H, Dahlén T, Khan A, Edgren G, Rzhetsky A. Measurable health effects associated with the daylight saving time shift. *PLoS Comput Biol.* 2020;16:e1007927. <https://doi.org/10.1371/journal.pcbi.1007927>.
 56. Roenneberg T, Winnebeck EC, Klerman EB. Daylight Saving Time and artificial time zones – a battle between biological and social times. *Front Physiol.* 2019;10:944. <https://doi.org/10.3389/fphys.2019.00944>.
 57. Hadlow NC, Brown S, Wardrop R, Henley D. The effects of season, daylight saving and time of sunrise on serum cortisol in a large population. *Chronobiol Int.* 2014;31:243–251. <https://doi.org/10.3109/07420528.2013.844162>.
 58. Yue F, Xia K, Wei L, et al. Effects of constant light exposure on sphingolipidomics and progression of NASH in high-fat-fed rats. *J Gastroenterol Hepatol.* 2020;35:1978–1989. <https://doi.org/10.1111/jgh.15005>.
 59. Shu YY, Gao WK, Chu HK, Yang L, Pan XL, Ye J. Attenuation by time-restricted feeding of high-fat and high-fructose diet-induced NASH in mice is related to Per2 and ferroptosis. *Oxid Med Cell Longev.* 2022;2022:8063897. <https://doi.org/10.1155/2022/8063897>.
 60. Guan D, Bae H, Zhou D, et al. Hepatocyte SREBP signaling mediates clock communication within the liver. *J Clin Invest.* 2023;133:e163018. <https://doi.org/10.1172/JCI163018>.
 61. Griffett K, Bedia-Diaz G, Elgendy B, Burris TP. REV-ERB agonism improves liver pathology in a mouse model of NASH. *PLoS One.* 2020;15:e0236000. <https://doi.org/10.1371/journal.pone.0236000>.
 62. Marjot J, Ray DW, Tomlinson JW. Is it time for chronopharmacology in NASH? *J Hepatol.* 2022;76:1215–1224. <https://doi.org/10.1016/j.jhep.2021.12.039>.
 63. Prosser RA, Glass JD. Assessing ethanol's actions in the suprachiasmatic circadian clock using in vivo and in vitro approaches. *Alcohol.* 2015;49:321–339. <https://doi.org/10.1016/j.alcohol.2014.07.016>.
 64. Filiano AN, Millender-Swain T, Johnson R Jr, Young ME, Gamble KL, Bailey SM. Chronic ethanol consumption disrupts the core molecular clock and diurnal rhythms of metabolic genes in the liver without affecting the suprachiasmatic nucleus. *PLoS One.* 2013;8:e71684. <https://doi.org/10.1371/journal.pone.0071684>.
 65. Gaucher J, Kinouchi K, Ceglia N, et al. Distinct metabolic adaptation of liver circadian pathways to acute and chronic patterns of alcohol intake. *Proc Natl Acad Sci U S A.* 2019;116:25250–25259. <https://doi.org/10.1073/pnas.1911189116>.
 66. Valcin JA, Udoh US, Swain TM, et al. Alcohol and liver clock disruption increase small droplet macrosteatosis, alter lipid metabolism and clock gene mRNA rhythms, and remodel the triglyceride lipidome in mouse liver. *Front Physiol.* 2020;11:1048. <https://doi.org/10.3389/fphys.2020.01048>.
 67. Meyrel M, Rolland B, Geoffroy PA. Alterations in circadian rhythms following alcohol use: a systematic review. *Prog Neuropsychopharmacol Biol Psychiatry.* 2020;99:109831. <https://doi.org/10.1016/j.pnpbp.2019.109831>.
 68. Ozburn AR, Falcon E, Mukherjee S, et al. The role of clock in ethanol-related behaviors. *Neuropsychopharmacology.* 2013;38:2393–2400. <https://doi.org/10.1038/npp.2013.138>.
 69. Rizk AA, Jenkins BW, Al-Sabagh Y, et al. The impact of sex, circadian disruption, and the clock^{Δ19/Δ19} genotype on alcohol drinking in mice. *Genes (Basel).* 2022;13:701. <https://doi.org/10.3390/genes13040701>.
 70. Summa KC, Jiang P, Fitzpatrick K, et al. Chronic alcohol exposure and the circadian clock mutation exert tissue-specific effects on gene expression in mouse hippocampus, liver, and proximal colon. *Alcohol Clin Exp Res.* 2015;39:1917–1929. <https://doi.org/10.1111/acer.12834>.
 71. de Zavalía N, Schoettner K, Goldsmith JA, et al. Bmal1 in the striatum influences alcohol intake in a sexually dimorphic manner. *Commun Biol.* 2021;4:1227. <https://doi.org/10.1038/s42003-021-02715-9>.
 72. Hug E, Winzeler K, Pfaltz MC, Cajochen C, Bader K. Later chronotype is associated with higher alcohol consumption and more adverse childhood experiences in young healthy women. *Clocks Sleep.* 2019;1:126–139. <https://doi.org/10.3390/clocks1sleep1010012>.
 73. Taylor BJ, Bowman MA, Brindle A, et al. Evening chronotype, alcohol use disorder severity, and emotion regulation in college students. *Chronobiol Int.* 2020;37:1725–1735. <https://doi.org/10.1080/07420528.2020.1800028>.
 74. Hasler BP, Sitnick SL, Shaw DS, Forbes EE. An altered neural response to reward may contribute to alcohol problems among late adolescents with an evening chronotype. *Psychiatry Res.* 2013;214:357–364. <https://doi.org/10.1016/j.psychres.2013.08.005>.
 75. Nowakowska-Domagala K, Mokros Ł, Jabłkowska-Górecka K, Grzelińska J, Pietras T. The relationship between chronotype and personality among patients with alcohol dependence syndrome: pilot study. *Chronobiol Int.* 2016;33:1351–1358. <https://doi.org/10.1080/07420528.2016.1213738>.

76. You M, Arteel GE. Effect of ethanol on lipid metabolism. *J Hepatol*. 2019;70:237–248. <https://doi.org/10.1016/j.jhep.2018.10.037>.
77. Zhang D, Tong X, Nelson BB, et al. The hepatic BMAL1/AKT/lipogenesis axis protects against alcoholic liver disease in mice via promoting PPAR α pathway. *Hepatology*. 2018;68:883–896. <https://doi.org/10.1002/hep.29878>.
78. Zhao Y, Zhang R, Wang Z, et al. Melatonin prevents against ethanol-induced liver injury by mitigating ferroptosis via targeting brain and muscle ARNT-like 1 in mice liver and HepG2 cells. *J Agric Food Chem*. 2022;70:12953–12967. <https://doi.org/10.1021/acs.jafc.2c04337>.
79. Zhou Y, Wu M, Xu L, et al. Bmal1 regulates macrophage polarize through glycolytic pathway in alcoholic liver disease. *Front Pharmacol*. 2021;12:640521. <https://doi.org/10.3389/fphar.2021.640521>.
80. Loftus EV Jr, Harewood GC, Loftus CG, et al. PSC-IBD: a unique form of inflammatory bowel disease associated with primary sclerosing cholangitis. *Gut*. 2005;54:91–96. <https://doi.org/10.1136/gut.2004.046615>.
81. de Vries AB, Janse M, Blokzijl H, Weersma RK. Distinctive inflammatory bowel disease phenotype in primary sclerosing cholangitis. *World J Gastroenterol*. 2015;21:1956–1971. <https://doi.org/10.3748/wjg.v21.i6.1956>.
82. Chen P, Kakan X, Wang S, et al. Deletion of clock gene Per2 exacerbates cholestatic liver injury and fibrosis in mice. *Exp Toxicol Pathol*. 2013;65:427–432. <https://doi.org/10.1016/j.etp.2011.12.007>.
83. Turco M, Cazzagon N, Franceschet I, et al. Morning bright light treatment for sleep-wake disturbances in primary biliary cholangitis: a pilot study. *Front Physiol*. 2018;9:1530. <https://doi.org/10.3389/fphys.2018.01530>.
84. Wu N, Carpino G, Ceci L, et al. Melatonin receptor 1A, but not 1B, knockout decreases biliary damage and liver fibrosis during cholestatic liver injury. *Hepatology*. 2022;75:797–813. <https://doi.org/10.1002/hep.32233>.
85. Chen L, Zhou T, Wu N, et al. Pinelectomy or light exposure exacerbates biliary damage and liver fibrosis in cholestatic rats through decreased melatonin synthesis. *Biochim Biophys Acta Mol Basis Dis*. 2019;1865:1525–1539. <https://doi.org/10.1016/j.bbdis.2019.03.002>.
86. Esmaili A, Nassiri Toosi M, Taher M, et al. A pilot randomized, clinical trial of the anti-pruritus effect of melatonin in patients with chronic liver disease. *Iran J Pharm Res*. 2021;20:462–472. <https://doi.org/10.22037/ijpr.2020.112942.14024>.
87. Huang DQ, Mathurin P, Cortez-Pinto H, Loomba R. Global epidemiology of alcohol-associated cirrhosis and HCC: trends, projections and risk factors. *Nat Rev Gastroenterol Hepatol*. 2023;20:37–49. <https://doi.org/10.1038/s41575-022-00688-6>.
88. Huang DQ, Singal AG, Kono Y, Tan DJH, El-Serag HB, Loomba R. Changing global epidemiology of liver cancer from 2010 to 2019: NASH is the fastest growing cause of liver cancer. *Cell Metab*. 2022;34:969–977 (e2). <https://doi.org/10.1016/j.cmet.2022.05.003>.
89. Ozakyol A. Global epidemiology of hepatocellular carcinoma (HCC epidemiology). *J Gastrointest Cancer*. 2017;48:238–240. <https://doi.org/10.1007/s12029-017-9959-0>.
90. IARC Monographs Vol 124 group. Carcinogenicity of night shift work. *Lancet Oncol*. 2019;20:1058–1059. [https://doi.org/10.1016/S1470-2045\(19\)30455-3](https://doi.org/10.1016/S1470-2045(19)30455-3).
91. VoPham T, Weaver MD, Vetter C, et al. Circadian misalignment and hepatocellular carcinoma incidence in the United States. *Cancer Epidemiol Biomarkers Prev*. 2018;27:719–727. <https://doi.org/10.1158/1055-9965.EPI-17-1052>.
92. Gu F, Xu S, Devesa SS, et al. Longitude position in a time zone and cancer risk in the United States. *Cancer Epidemiol Biomarkers Prev*. 2017;26:1306–1311. <https://doi.org/10.1158/1055-9965.EPI-16-1029>.
93. Liang Y, Wang S, Huang X, et al. Dysregulation of circadian clock genes as significant clinic factor in the tumorigenesis of hepatocellular carcinoma. *Comput Math Methods Med*. 2021;2021:8238833. <https://doi.org/10.1155/2021/8238833>.
94. Liu H, Gao Y, Hu S, Fan Z, Wang X, Li S. Bioinformatics analysis of differentially expressed rhythm genes in liver hepatocellular carcinoma. *Front Genet*. 2021;12:680528. <https://doi.org/10.3389/fgene.2021.680528>.
95. Kettner NM, Voicu H, Finegold MJ, et al. Circadian homeostasis of liver metabolism suppresses hepatocarcinogenesis. *Cancer Cell*. 2016;30:909–924. <https://doi.org/10.1016/j.ccell.2016.10.007>.
96. Qu M, Zhang G, Qu H, et al. Circadian regulator BMAL1::CLOCK promotes cell proliferation in hepatocellular carcinoma by controlling apoptosis and cell cycle. *Proc Natl Acad Sci U S A*. 2023;120:e2214829120. <https://doi.org/10.1073/pnas.2214829120>.
97. Mteyrek A, Filipski E, Guettier C, Okyar A, Lévi F. Clock gene Per2 as a controller of liver carcinogenesis. *Oncotarget*. 2016;7:85832–85847. <https://doi.org/10.18632/oncotarget.11037>.
98. Reiter RJ, Mayo JC, Tan DX, Sainz RM, Alatorre-Jimenez M, Qin L. Melatonin as an antioxidant: under promises but over delivers. *J Pineal Res*. 2016;61:253–278. <https://doi.org/10.1111/jpi.12360>.
99. El-Magd MA, Mohamed Y, El-Shetry ES, et al. Melatonin maximizes the therapeutic potential of non-preconditioned MSCs in a DEN-induced rat model of HCC. *Biomed Pharmacother*. 2019;114:108732. <https://doi.org/10.1016/j.biopha.2019.108732>.
100. Sánchez DI, González-Fernández B, Crespo I, et al. Melatonin modulates dysregulated circadian clocks in mice with diethylnitrosamine-induced hepatocellular carcinoma. *J Pineal Res*. 2018;65:e12506. <https://doi.org/10.1111/jpi.12506>.
101. Su SC, Ho YC, Liu YF, et al. Association of melatonin membrane receptor 1A/1B gene polymorphisms with the occurrence and metastasis of hepatocellular carcinoma. *Oncotarget*. 2017;8:85655–85669. <https://doi.org/10.18632/oncotarget.21107>.
102. GBD 2017 Inflammatory Bowel Disease Collaborators. The global, regional, and national burden of inflammatory bowel disease in 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet Gastroenterol Hepatol*. 2020;5:17–30. [https://doi.org/10.1016/S2468-1253\(19\)30333-4](https://doi.org/10.1016/S2468-1253(19)30333-4).
103. Le Berre C, Ananthakrishnan AN, Danese S, Singh S, Peyrin-Biroulet L. Ulcerative colitis and Crohn's disease have similar burden and goals for treatment. *Clin Gastroenterol Hepatol*. 2020;18:14–23. <https://doi.org/10.1016/j.cgh.2019.07.005>.
104. Ni Y, Zhao Y, Ma L, et al. Pharmacological activation of REV-ERB α improves nonalcoholic steatohepatitis by regulating intestinal permeability. *Metabolism*. 2021;114:154409. <https://doi.org/10.1016/j.metabol.2020.154409>.
105. Thaïs CA, Levy M, Korem T, et al. Microbiota diurnal rhythmicity programs host transcriptome oscillations. *Cell*. 2016;167:1495–1510 (e12). <https://doi.org/10.1016/j.cell.2016.11.003>.
106. Liang X, Bushman FD, FitzGerald GA. Rhythmicity of the intestinal microbiota is regulated by gender and the host circadian clock. *Proc Natl Acad Sci U S A*. 2015;112:10479–10484. <https://doi.org/10.1073/pnas.1501305112>.
107. Zarrinpar A, Chaix A, Yooseph S, Panda S. Diet and feeding pattern affect the diurnal dynamics of the gut microbiome. *Cell Metab*. 2014;20:1006–1017. <https://doi.org/10.1016/j.cmet.2014.11.008>.
108. Nojkov B, Rubenstein JH, Chey WD, Hoogerwerf WA. The impact of rotating shift work on the prevalence of irritable bowel syndrome in nurses. *Am J Gastroenterol*. 2010;105:842–847. <https://doi.org/10.1038/ajg.2010.48>.
109. Kim HI, Jung SA, Choi JY, et al. Impact of shiftwork on irritable bowel syndrome and functional dyspepsia. *J Korean Med Sci*. 2013;28:431–437. <https://doi.org/10.3346/jkms.2013.28.3.431>.
110. Yuan S, Sun Y, Tan X, et al. Sleep duration and daytime napping in relation to incident inflammatory bowel disease: a prospective cohort study. *Aliment Pharmacol Ther*. 2023;57:475–485. <https://doi.org/10.1111/apt.17285>.
111. Chakradeo PS, Keshavarzian A, Singh S, et al. Chronotype, social jet lag, sleep debt and food timing in inflammatory bowel disease. *Sleep Med*. 2018;52:188–195. <https://doi.org/10.1016/j.sleep.2018.08.002>.
112. van Langenberg DR, Papandony MC, Gibson PR. Sleep and physical activity measured by accelerometry in Crohn's disease. *Aliment Pharmacol Ther*. 2015;41:991–1004. <https://doi.org/10.1111/apt.13160>.
113. Hillemecher T, Bachmann O, Kahl KG, Frieling H. Alcohol, microbiome, and their effect on psychiatric disorders. *Prog Neuropsychopharmacol Biol Psychiatry*. 2018;85:105–115. <https://doi.org/10.1016/j.pnpbp.2018.04.015>.
114. Mendes BG, Schnabl B. From intestinal dysbiosis to alcohol-associated liver disease. *Clin Mol Hepatol*. 2020;26:595–605. <https://doi.org/10.3350/cmh.2020.0086>.
115. Chen P, Stärkel P, Turner JR, Ho SB, Schnabl B. Dysbiosis-induced intestinal inflammation activates tumor necrosis factor receptor I and mediates alcoholic liver disease in mice. *Hepatology*. 2015;61:883–894. <https://doi.org/10.1002/hep.27489>.
116. Voigt RM, Summa KC, Forsyth CB, et al. The circadian clock mutation promotes intestinal dysbiosis. *Alcohol Clin Exp Res*. 2016;40:335–347. <https://doi.org/10.1111/acer.12943>.
117. Swanson GR, Siskin J, Gorenz A, et al. Disrupted diurnal oscillation of gut-derived short chain fatty acids in shift workers drinking alcohol: possible mechanism for loss of resiliency of intestinal barrier in disrupted circadian host. *Transl Res*. 2020;221:97–109. <https://doi.org/10.1016/j.trsl.2020.04.004>.
118. Swanson GR, Gorenz A, Shaikh M, et al. Night workers with circadian misalignment are susceptible to alcohol-induced intestinal hyperpermeability with social drinking. *Am J Physiol Gastrointest Liver Physiol*. 2016;311:G192–G201. <https://doi.org/10.1152/ajpgi.00087.2016>.
119. Swanson GR, Gorenz A, Shaikh M, et al. Decreased melatonin secretion is associated with increased intestinal permeability and marker of endotoxemia in alcoholics. *Am J Physiol Gastrointest Liver Physiol*. 2015;308:G1004–G1011. <https://doi.org/10.1152/ajpgi.00002.2015>.
120. Zhao K, Ni Z, Qin Y, et al. Disrupted diurnal oscillations of the gut microbiota in patients with alcohol dependence. *Front Cell Infect Microbiol*. 2023;13:1127011. <https://doi.org/10.3389/fcimb.2023.1127011>.
121. Dubinkina VB, Tyakht AV, Odintsova VY, et al. Links of gut microbiota composition with alcohol dependence syndrome and alcoholic liver disease. *Microbiome*. 2017;5:141. <https://doi.org/10.1186/s40168-017-0359-2>.
122. Hermida RC, Crespo JJ, Domínguez-Sardiña M, et al. Bedtime hypertension treatment improves cardiovascular risk reduction: the Hygia Chronotherapy Trial. *Eur Heart J*. 2020;41:4565–4576. <https://doi.org/10.1093/eurheartj/ehz754>.
123. Damato AR, Herzog ED. Circadian clock synchrony and chronotherapy opportunities in cancer treatment. *Semin Cell Dev Biol*. 2022;126:27–36. <https://doi.org/10.1016/j.semcdb.2021.07.017>.
124. Cunningham JEA, Stamp JA, Shapiro CM. Sleep and major depressive disorder: a review of non-pharmacological chronotherapeutic treatments for unipolar depression. *Sleep Med*. 2019;61:6–18. <https://doi.org/10.1016/j.sleep.2019.04.012>.
125. Kaur G, Phillips CL, Wong K, McLachlan AJ, Saini B. Timing of administration: for commonly-prescribed medicines in Australia. *Pharmaceutics*. 2016;8:13. <https://doi.org/10.3390/pharmaceutics8020013>.

126. Ye Y, Xu H, Xie Z, et al. Time-restricted feeding reduces the detrimental effects of a high-fat diet, possibly by modulating the circadian rhythm of hepatic lipid metabolism and gut microbiota. *Front Nutr*. 2020;7:596285. <https://doi.org/10.3389/fnut.2020.596285>.
127. Wilson RB, Zhang R, Chen YJ, et al. Two-week isocaloric time-restricted feeding decreases liver inflammation without significant weight loss in obese mice with non-alcoholic fatty liver disease. *Int J Mol Sci*. 2020;21:9156. <https://doi.org/10.3390/ijms21239156>.
128. Hua L, Li J, Yang Y, et al. Liver-derived FGF21 is required for the effect of time-restricted feeding on high-fat diet-induced fatty liver in mice. *FASEB J*. 2023;37:e22898. <https://doi.org/10.1096/fj.202202031R>.
129. Cienfuegos S, Gabel K, Kalam F, et al. Effects of 4- and 6-h time-restricted feeding on weight and cardiometabolic health: a randomized controlled trial in adults with obesity. *Cell Metab*. 2020;32:366–378 (e3). <https://doi.org/10.1016/j.cmet.2020.06.018>.
130. McAllister MJ, Pigg BL, Renteria LI, Waldman HS. Time-restricted feeding improves markers of cardiometabolic health in physically active college-age men: a 4-week randomized pre-post pilot study. *Nutr Res*. 2020;75:32–43. <https://doi.org/10.1016/j.nutres.2019.12.001>.
131. Kord-Varkaneh H, Salehi-Sahlabadi A, Tinsley GM, Santos HO, Hekmatdoost A. Effects of time-restricted feeding (16/8) combined with a low-sugar diet on the management of non-alcoholic fatty liver disease: a randomized controlled trial. *Nutrition*. 2023;105:111847. <https://doi.org/10.1016/j.nut.2022.111847>.
132. Gaucher J, Montellier E, Sassone-Corsi P. Molecular cogs: interplay between circadian clock and cell cycle. *Trends Cell Biol*. 2018;28:368–379. <https://doi.org/10.1016/j.tcb.2018.01.006>.
133. Lévi F, Zidani R, Misser JL. Randomised multicentre trial of chronotherapy with oxaliplatin, fluorouracil, and folinic acid in metastatic colorectal cancer. *International Organization for Cancer Chronotherapy*. *Lancet*. 1997;350:681–686. [https://doi.org/10.1016/s0140-6736\(97\)03358-8](https://doi.org/10.1016/s0140-6736(97)03358-8).
134. Albuquerque T, Neves AR, Quintela T, Costa D. Exploring the link between chronobiology and drug delivery: effects on cancer therapy. *J Mol Med (Berl)*. 2021;99:1349–1371. <https://doi.org/10.1007/s00109-021-02106-x>.
135. Marin JG, Macias RIR, Monte MJ, et al. Molecular bases of drug resistance in hepatocellular carcinoma. *Cancers (Basel)*. 2020;12:1663. <https://doi.org/10.3390/cancers12061663>.
136. Hassan SA, Schmithals C, von Harten M, Piiper A, Korf HW, von Gall C. Time-dependent changes in proliferation, DNA damage and clock gene expression in hepatocellular carcinoma and healthy liver of a transgenic mouse model. *Int J Cancer*. 2021;148:226–237. <https://doi.org/10.1002/ijc.33228>.
137. Hassan SA, Ali AAH, Sohn D, et al. Does timing matter in radiotherapy of hepatocellular carcinoma? An experimental study in mice. *Cancer Med*. 2021;10:7712–7725. <https://doi.org/10.1002/cam4.4277>.
138. Fernández-Palanca P, Méndez-Blanco C, Fondevila F, et al. Melatonin as an antitumor agent against liver cancer: an updated systematic review. *Antioxidants (Basel)*. 2021;10:103. <https://doi.org/10.3390/antiox10010103>.
139. Prieto-Domínguez N, Ordóñez R, Fernández A, et al. Melatonin-induced increase in sensitivity of human hepatocellular carcinoma cells to sorafenib is associated with reactive oxygen species production and mitophagy. *J Pineal Res*. 2016;61:396–407. <https://doi.org/10.1111/jpi.12358>.
140. Zhou B, Lu Q, Liu J, et al. Melatonin increases the sensitivity of hepatocellular carcinoma to sorafenib through the PERK-ATF4-Beclin1 pathway. *Int J Biol Sci*. 2019;15:1905–1920. <https://doi.org/10.7150/ijbs.32550>.
141. Moreira AJ, Ordoñez R, Cerski CT, et al. Melatonin activates endoplasmic reticulum stress and apoptosis in rats with diethylnitrosamine-induced hepatocarcinogenesis. *PLoS One*. 2015;10:e0144517. <https://doi.org/10.1371/journal.pone.0144517>.
142. Pakravan H, Ahmadian M, Fani A, Aghaee D, Brumanad S, Pakzad B. The effects of melatonin in patients with nonalcoholic fatty liver disease: a randomized controlled trial. *Adv Biomed Res*. 2017;6:40. <https://doi.org/10.4103/2277-9175.204593>.
143. Akhavan Rezayat A, Ghasemi Nour M, Bondarsahebi Y, et al. The effects of melatonin therapy on the treatment of patients with non-alcoholic steatohepatitis: a systematic review and meta-analysis on clinical trial studies. *Eur J Pharmacol*. 2021;905:174154. <https://doi.org/10.1016/j.ejphar.2021.174154>.
144. Bahrami M, Cheraghpour M, Jafarirad S, et al. The effect of melatonin on treatment of patients with non-alcoholic fatty liver disease: a randomized double blind clinical trial. *Complement Ther Med*. 2020;52:102452. <https://doi.org/10.1016/j.ctim.2020.102452>.
145. To H, Yoshimatsu H, Tomonari M, et al. Methotrexate chronotherapy is effective against rheumatoid arthritis. *Chronobiol Int*. 2011;28:267–274. <https://doi.org/10.3109/07420528.2011.553017>.
146. Scherholz ML, Schlesinger N, Androulakis IP. Chronopharmacology of glucocorticoids. *Adv Drug Deliv Rev*. 2019;151–152:245–261. <https://doi.org/10.1016/j.addr.2019.02.004>.
147. Swanson GR, Biglin M, Raff H, et al. Impact of chronotherapy on 6-mercaptopurine metabolites in inflammatory bowel disease: a pilot cross-over trial. *Clin Transl Gastroenterol*. 2023;14:e00549. <https://doi.org/10.14309/ctg.0000000000000549>.
148. Zhou Z, Lin Y, Gao L, Yang Z, Wang S, Wu B. Circadian pharmacological effects of berberine on chronic colitis in mice: role of the clock component Rev-erb α . *Biochem Pharmacol*. 2020;172:113773. <https://doi.org/10.1016/j.bcp.2019.113773>.
149. Kim SW, Kim S, Son M, Cheon JH, Park YS. Melatonin controls microbiota in colitis by goblet cell differentiation and antimicrobial peptide production through Toll-like receptor 4 signalling. *Sci Rep*. 2020;10:2232. <https://doi.org/10.1038/s41598-020-59314-7>.
150. Zhao ZX, Yuan X, Cui YY, et al. Melatonin mitigates oxazolone-induced colitis in microbiota-dependent manner. *Front Immunol*. 2022;12:783806. <https://doi.org/10.3389/fimmu.2021.783806>.
151. Voigt RM, Forsyth CB, Keshavarzian A. Circadian rhythms: a regulator of gastrointestinal health and dysfunction. *Expert Rev Gastroenterol Hepatol*. 2019;13:411–424. <https://doi.org/10.1080/17474124.2019.1595588>.
152. Zhang R, Lahens NF, Ballance HI, Hughes ME, Hogenesch JB. A circadian gene expression atlas in mammals: implications for biology and medicine. *Proc Natl Acad Sci U S A*. 2014;111:16219–16224. <https://doi.org/10.1073/pnas.1408886111>.